Scientific Abstract

This is a pilot study to evaluate the safety and feasibility of gene therapy to correct the genetic defect in the hematopoietic cells of patients with Fanconi Anemia. This study will incorporate recent technological advances, which have significantly improved gene transfer efficiency in preclinical models. The combination of G-CSF and SCF mobilization of peripheral blood stem cells combined with transduction protocols which include fibronectin and cytokine stimulation have resulted in up to 20% of the hematopoietic cells containing the transferred gene. Baseline studies will include a bone marrow analysis to ensure that chromosomal abnormalities are not present prior to reinfusion of the genetically manipulated cells. Patients with Fanconi Anemia complementation groups A and C will have peripheral blood stem cells mobilized with the cytokine combination of G-CSF and SCF until a target count of 6 CD34+ cells/mm^3 is achieved. Patients who do not achieve this target count by 15 days will be off study. If more than 30% of the patients have grade III-IV toxicity associated with the cytokine combination the study will stop. Patients who achieve a target count of 6 CD34+ cells/mm^3 will undergo apheresis for collection of peripheral blood stem cells. The cytokine combination will continue to be administered during apheresis. A target collection of $\geq 1 \times 10^6$ CD34+ cells/kg will be obtained over a maximum of 10 days of apheresis. The peripheral blood stem cells will be cryopreserved. If less than 30% of the patients do not mobilize adequate peripheral blood stem cells the protocol will stop. A bone marrow analysis will be performed 14 days after discontinuing the cytokines to characterize the ability of the cytokines to activate the hematopoietic stem cells for retroviral transduction.

The cryopreserved peripheral blood stem cells will be pooled and CD34+ cells will be isolated using the ISOLEX® 300i system developed by Nexell Therapeutics Inc. The CD34+ cells will be transduced with a retrovirus construct carrying either the Fanconi Anemia complementation group A or C cDNA for patients with the respective molecular defect. The genetically modified peripheral blood stem cells will be reinfused approximately one month after apheresis is complete. If greater than 30% of the patients have grade III-IV toxicity associated with the reinfusion the study will be stopped. Transfer of the retroviral construct will be analyzed sequentially from the peripheral blood on days 7, 14, 21, 28, 42 and 56 and from a bone marrow analysis 28 days from the infusion. If positive, testing of peripheral blood will continue monthly until transduced cells are not detectable on 3 determinations and a bone marrow will be obtained at day 84. Safety testing for the presence of replication competent retrovirus should be done on peripheral blood monthly for 3 months and every 3 months up to 1 year and then yearly. If there is no gene transfer in the first five patients the study will be stopped. If there is evidence of a replication competent retrovirus in one patient the study will be stopped.